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Section 29

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PATENT PEQUEST : STANDARD PATENT

I/We, being the person/s identified below as the Applicant, request the grant of a patent to the person/s indicated below as the Nominated Person/s, for an invention described in the accompanying standard complete specification.

Full application details follow.

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Lineare adhasionsinhibitoren

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(57) Claim

1. Linear peptides of the formula I

X-A-B-C-Arg-E-G-L-Z I,

in which

X is H, acyl having 1-10 C atoms, H-Asn, H-Val-Asn, H-Asp-Val-Asn, Fmoc-Gly-Gly, H-Lys-Gly-Gly, H-Lys-Pro, H-Tyr-Gly-Gly, H-Cys-Gly-Gly, H-Cys(Trt)-Gly-Gly, H-Cys-Gly-Gly-Thr-Asp-Val-Asn or H-Thr-Asp-Val-Asn,

A, B and C in each case independently of one another are absent or are in each case an amino acid radical, selected from a group consisting of Ala, Arg, Asn, Asp, Asp(OR), Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Lys(Ac), Lys($AcNH_2$), Lys(AcSH), Met, Orn, Phe, 4-Hal-Phe, Pro, Ser, Thr, Trp, Tyr or Val, where the amino acid radicals mentioned can also be derivatized,

E is Gly, His or Leu-His,

.../2

G is absent or is Asp or Asn,
L is absent or is Gly, Ile, Leu or Leu-Leu,
Z is NH_2 or OH,
Hal is F, Cl, Br or I and
Ac is alkanoyl having 1-10 C atoms,
and their physiologically acceptable salts.

7. Use of compounds of the formula I according to Claim 1 or of their physiologically acceptable salts for the production of a medicament for the control of diseases.

10. Use of compounds of the formula I according to Claim 1 for the purification of integrins by affinity chromatography.

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AUSTRALIA

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ORIGINAL
COMPLETE SPECIFICATION
STANDARD PATENT

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Invention Title: Lineare adhasionsinhibitoren

The following statement is a full description of this invention, including the best method of performing it known to me:-

5020

Linear adhesion inhibitors

The invention relates to novel linear peptides of the formula I

X-A-B-C-Arg-E-G-L-Z I,

in which

X is H, acyl having 1-10 C atoms, H-Asn, H-Val-Asn, H-Asp-Val-Asn, Fmoc-Gly-Gly, H-Lys-Gly-Gly, H-Lys-Pro, H-Tyr-Gly-Gly, H-Cys-Gly-Gly, H-Cys(Trt)-Gly-Gly, H-Cys-Gly-Gly-Thr-Asp-Val-Asn or H-Thr-Asp-Val-Asn,

A, B and C in each case independently of one another are absent or are an amino acid radical, selected from a group consisting of Ala, Arg, Asn, Asp, Asp(OR), Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Lys(Ac), Lys($AcNH_2$), Lys(AcSH), Met, Orn, Phe, 4-Hal-Phe, Pro, Ser, Thr, Trp, Tyr or Val, where the amino acid radicals mentioned can also be derivatized,

E is Gly, His or Leu-His,

G is absent or is Asp or Asn,

L is absent or is Gly, Ile, Leu or Leu-Leu,

Z is NH_2 or OH,

Hal is F, Cl, Br or I and

Ac is alkanoyl having 1-10 C atoms,

and their physiologically acceptable salts.

Similar compounds are known, for example, from European Patent Application EP 0 406 428.

The object of the invention was to find novel compounds having useful properties, in particular those which can be used for the production of medicaments.

It has been found that the compounds of the formula I and their salts have very useful properties. In particular, they act as integrin inhibitors, in which case they particularly inhibit the interactions of β_3-integrin receptors with ligands. This action can be

demonstrated, for example, by the method which is described by J. W. Smith et al. in J. Biol. Chem. 265, 12267-12271 (1990). In addition, there are anti-inflammatory effects. This action can also be demonstrated with the aid of methods which are known from the literature.

The compounds can be employed as pharmaceutical active compounds in human and veterinary medicine, in particular for the prophylaxis and the treatment of disorders of the circulation, in thrombosis, cardiac infarct, arteriosclerosis, inflammations, apoplexy, angina pectoris, tumours, osteolytic disorders, in particular osteoporosis, angiogenesis and restenosis after angioplasty. They can also be used in a supportive role in wound-healing processes.

The compounds are also suitable as antimicrobial agents which avoid infections as they were caused for example by bacteria, fungi or yeasts. The substances are useful as accompanying antimicrobial agents in cases where operations are effected in order to insert non-corporal materials, for example such as biomaterials, implants, catheters or heart-pacemakers. They act as antiseptics.

The abbreviations of amino acid radicals shown above and below stand for the radicals of the following amino acids:

Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Asp(OR)	aspartic acid (β-ester)
Cit	citrulline
Cys	cysteine
Dab	2,4-diaminobutyric acid
Gln	glutamine
Glu	glutamic acid
Gly	glycine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Lys(Ac)	N$^{\epsilon}$-acyllysine
Lys($AcNH_2$)	N$^{\epsilon}$-aminoacyllysine
Lys(AcSH)	N$^{\epsilon}$-mercaptoacyllysine
Met	methionine
Orn	ornithine
Phe	phenylalanine

4-Hal-Phe	4-halophenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine.

In addition, the following have the meanings below:

BOC	tert-butoxycarbonyl
CBZ	benzyloxycarbonyl
DCCI	dicyclohexylcarbodiimide
DMF	dimethylformamide
EDCI	N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride
Et	ethyl
FMOC	9-fluorenylmethoxycarbonyl
HOBt	1-hydroxybenzotriazole
Me	methyl
MBHA	4-methylbenzhydrylamine
Mtr	4-methoxy-2,3,6-trimethylphenylsulfonyl
OBut	tert-butyl ester
OMe	methyl ester
OEt	ethyl ester
POA	phenoxyacetyl
TFA	trifluoroacetic acid
Trt	trityl (triphenylmethyl).

If the amino acids mentioned above can occur in several enantiomeric forms, then all these forms and also their mixtures (e.g. the DL-forms) are included above and below, e.g. as constituents of the compounds of the formula I. The amino acids and/or the amino acid radicals can also be derivatized in a form known per se.

The invention further relates to a process for the preparation of a compound of the formula I according to Claim 1 or of one of its salts, characterized in that it is liberated from one of its functional derivatives by treating with a solvolysing or hydrogenolysing agent, or in that a peptide of the formula II

M-OH II

in which

M is (a) X, but not hydrogen, Fmoc-Gly, H-Lys-Gly, H-Lys, H-Tyr-Gly, H-Asp-Val, H-Tyr, H-Val, H-Asp, H-Cys-Gly-Gly-Thr-Asp-Val, H-Cys-Gly-Gly-Thr-Asp, H-Cys-Gly-Gly-Thr, H-Cys-Gly-Gly, H-Cys-Gly, H-Cys, H-Cys(Trt)-Gly, H-Cys(Trt), H-Thr-Asp-Val, H-Thr-Asp or H-Thr,

(b) X-A,

(c) X-A-B,

(d) X-A-B-C,

(e) X-A-B-C-Arg,

(f) X-A-B-C-Arg-E or

(g) X-A-B-C-Arg-E-G

is reacted with an amino compound of the formula III

H-Q-Z III

in which Z has the meaning indicated and

Q is (a) A-B-C-Arg-E-G-L, Asn-A-B-C-Arg-E-G-L Val-Asn-A-B-C-Arg-E-G-L, Pro-A-B-C-Arg-E-G-L, Gly-A-B-C-Arg-E-G-L, Gly-Gly-A-B-C-Arg-E-G-L, Asn-A-B-C-Arg-E-G-L, Val-Asn-A-B-C-Arg-E-G-L Asp-Val-Asn-A-B-C-Arg-E-G-L, Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L, Gly-Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L or Gly-Gly-Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L,

(b) B-C-Arg-E-G-L,

(c) C-Arg-E-G-L,

(d) Arg-E-G-L,

(e) E-G-L,

(f) G-L or

(g) L

and/or in that a free amino group is optionally acylated and/or a compound of the formula I is converted into one of its salts by treating with an acid or a base.

The radicals A, B, C, E, G, L, M, Q, X and Z above and below have the meanings indicated in the formulae I, II and III, if not expressly stated otherwise.

In the above formulae, alkyl is preferably methyl, ethyl, isopropyl or tert-butyl.

X is preferably hydrogen, H-Asn, Fmoc-Gly-Gly or H-Cys(Trt)-Gly-Gly, but particularly preferably H-Cys-Gly-Gly. In addition, X can also be acyl having 1-10 C atoms, acyl preferably being alkanoyl having 1-8, in particular 1, 2, 3 or 4 C atoms, specifically preferably formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl or hexanoyl, but also aryl-CO, such as e.g. benzoyl, o-, m- or p-toluyl, o-, m- or p-methoxybenzoyl and also 2-naphthoyl or else aryl-C_nH_{2n}-CO (n = 1-4), such as e.g. Ph-CH_2-CO, Ph$(CH_2)_2$-CO, Ph-$(CH_2)_3$CO or Ph-$(CH_2)_4$-CO (Ph = phenyl), where Ph can also be substituted by an OCH_3 or CH_3 group.

The group A is preferably Ala, Leu or Ser, in particular Gly, or else is not present. B is preferably Asn or Asp. C is preferably Ala, also D-Ala, His and in particular Gly. E is preferably Gly or His. G is preferably Asp, while L is preferably Leu. Z is NH_2, but particularly preferably OH.

Accordingly, the invention in particular relates to those compounds of the formula I in which at least one of the said radicals has one of the preferred meanings indicated above.

A preferred group of compounds can be expressed

by the formula Ia, which corresponds to the formula I and in which B, C, E, X and Z have the meanings indicated there and

A is Gly,
G is Asp and
L is Leu.

A further group of preferred compounds can be expressed by the part formulae Iaa to Iad, which otherwise correspond to the formula I or Ia, but in which

in Iaa:
- X is hydrogen or acetyl
- A is Gly,
- B is Asn or Asp,
- C is absent, is Ala or Gly,
- E is Gly or His,
- G is Asp,
- L is Leu and
- Z is NH_2 or OH,

in Iab:
- X is hydrogen or acyl,
- A is absent or is Gly,
- B is absent or is Asn,
- C is Gly,
- E is Gly or His,
- G is Asp,
- L is Leu and
- Z is NH_2 or OH,

in Iac:
- X is hydrogen,
- A is absent or is Gly,
- B is absent or is Asn,
- C is absent or is Gly,
- E is Gly or His,
- G is Asp,
- L is Leu and
- Z is OH or NH_2,

in Iad: X is H-Asn, H-Val-Asn, H-Asp-Val-Asn, Fmoc-Gly-Gly, H-Lys-Gly-Gly, H-Tyr-Gly-Gly, H-Cys(Trt)-Gly-Gly, H-Cys-Gly-Gly, H-Cys-Gly-Gly-Thr-Asp-Val-Asn or H-Thr-Asp-Val-Asn,

A is Gly,
B is Asn,
C is Gly,
E is Gly or His,
G is Asp,
L is Leu and
Z is NH_2 or OH.

Particularly suitable compounds are those which correspond to the formula I and in which A, B, C, X and Z have the abovementioned preferred meanings, but in which the central amino acid radical is L-Arg, not D-Arg, E is Gly and also His, not D-His, G is Asp and L is Leu.

The compounds of the formula I and also the starting materials for their preparation are otherwise prepared by known methods, as are described in the literature (e.g. in the standard works such as Houben-Weyl, Methoden der organischen Chemie, (Methods of Organic Chemistry) Georg-Thieme-Verlag, Stuttgart), in particular under reaction conditions which are known and suitable for the said reactions. In this context, use can also be made of known variants which are not mentioned in more detail here.

The starting substances can also be formed in situ, if desired, such that they are not isolated from the reaction mixture, but immediately reacted further to give the compounds of the formula I.

The compounds of the formula I can be obtained by liberating them from their functional derivatives by solvolysis, in particular hydrolysis, or by hydrogenolysis.

Preferred starting materials for the solvolysis or hydrogenolysis are those which contain appropriate protected amino and/or hydroxyl groups instead of one or

more free amino and/or hydroxyl groups, preferably those which carry an amino protecting group instead of an H atom which is bonded to an N atom, e.g. those which correspond to the formula I, but contain an NHR' group (in which R' is an amino protecting group, e.g. BOC or CBZ) instead of an NH_2 group.

In addition, starting materials are preferred which carry a hydroxyl protecting group instead of the H atom of a hydroxyl group, e.g. those which correspond to the formula I, but contain an R''O-phenyl group (in which R'' is a hydroxyl protecting group) instead of a hydroxyphenyl group.

Several - identical or different - protected amino and/or hydroxyl groups can be present in the molecule of the starting material. If the protective groups present are different from one another, in many cases they can be removed selectively.

The expression "amino protecting group" is generally known and relates to groups which are suitable for protecting (for blocking) an amino group from chemical reactions, but which are easily removable, after the desired chemical reaction has been carried out at other positions in the molecule. Typical groups of this type are, in particular, unsubstituted or substituted acyl, aryl, aralkoxymethyl or aralkyl groups. As the amino protecting groups are removed after the desired reaction (or reaction sequence), their nature and size is otherwise not critical; but those having 1-20, in particular 1-8, C atoms are preferred. The expression "acyl group" is to be taken in its widest sense in connection with the present process and the present compounds. It includes acyl groups derived from aliphatic, araliphatic, aromatic or heterocyclic carboxylic acids or sulfonic acids and in particular alkoxycarbonyl, aryloxycarbonyl and, in particular, aralkoxycarbonyl groups. Examples of acyl groups of this type are alkanoyl such as acetyl, propionyl or butyryl; aralkanoyl such as phenylacetyl; aroyl such as benzoyl or toluyl; aryloxyalkanoyl such as POA; alkoxycarbonyl such

as methoxycarbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, BOC, 2-iodoethoxycarbonyl; aralkyloxycarbonyl such as CBZ ("carbobenzoxy"), 4-methoxybenzyloxycarbonyl, FMOC, and arylsulfonyl such as Mtr. Preferred amino protecting groups are BOC and Mtr, and in addition CBZ, FMOC, benzyl and acetyl.

The expression "hydroxy protecting group" is also generally known and relates to groups which are suitable for protecting a hydroxyl group from chemical reactions, but which are easily removable, after the desired chemical reaction has been carried out at other positions in the molecule. Typical groups of this type are the above-mentioned unsubstituted or substituted aryl, aralkyl or acyl groups, and in addition also alkyl groups. The nature and size of the hydroxy protecting groups is not critical, as they are removed again after the desired chemical reaction or reaction sequence; preferred groups are those having 1-20, in particular 1-10 C atoms. Examples of hydroxyl protecting groups are, inter alia, benzyl, p-nitrobenzoyl, p-toluenesulfonyl and acetyl, benzyl and acetyl being particularly preferred. The COOH groups in aspartic acid and glutamic acid are preferably protected in the form of their tert-butyl esters (e.g. Asp (OBut)).

The functional derivatives of the compounds of the formula I to be used as starting materials can be prepared by customary methods of amino acid and peptide synthesis, such as are described e.g. in the said standard works and patent applications, and e.g. also by the Merrifield solid phase method (B.F. Gysin and R.B. Merrifield, J. Am. Chem. Soc. 94, 3102 et seq. (1972)).

The liberation of the compounds of the formula I from their functional derivatives is carried out - depending on the protecting group used - e.g. with strong acids, preferably with TFA or perchloric acid, but also with other strong inorganic acids such as hydrochloric acid or sulfuric acid, or strong organic carboxylic acids such as trichloroacetic acid or sulfonic acids such as benzene- or p-toluenesulfonic acid. The presence of an

additional inert solvent is possible, but not always necessary. Suitable inert solvents are preferably organic, for example carboxylic acids such as acetic acid, ethers such as tetrahydrofuran or dioxane, amides such as DMF, halogenated hydrocarbons such as dichloromethane, and in addition also alcohols such as methanol, ethanol or isopropanol and also water. In addition, mixtures of the abovementioned solvents are suitable. TFA is preferably used in an excess without addition of a further solvent, perchloric acid in the form of a mixture of acetic acid and 70 % perchloric acid in the ratio 9:1. The reaction temperatures for the cleavage are expediently between about 0 and about 50°, preferably between 15 and 30° (room temperature).

The groups BOC, OBut and Mtr can be removed e.g. preferably using TFA in dichloromethane or using about 3 to 5 N HCl in dioxane at 15-30°, the FMOC group using an about 5- to 50 % solution of dimethylamine, diethylamine or piperidine in DMF at 15-30°.

Protecting groups which can be removed by hydrogenolysis (e.g. CBZ or benzyl) can be removed, e.g. by treating with hydrogen in the presence of a catalyst (e.g. a noble metal catalyst such as palladium, preferably on a carrier such as carbon). Suitable solvents in this case are those indicated above, in particular e.g. alcohols such as methanol or ethanol or amides such as DMF. The hydrogenolysis is carried out, as a rule, at temperatures between about 0 and 100° and pressures between about 1 and 200 bar, preferably at 20-30° and 1-10 bar. Hydrogenolysis of the CBZ group is easily carried out e.g. on 5 to 10 % Pd-C in methanol or using ammonium formate (instead of H_2) on Pd-C in methanol/DMF at 20-30°.

Compounds of the formula I can also be obtained by reaction of a compound of the formula II under condensing conditions known per se for peptide syntheses, as are described e.g. in Houben-Weyl, loc cit. volume 15/II, pages 1 to 806 (1974).

The reaction is preferably carried out in the

presence of a dehydrating agent, e.g. a carbodiimide such as DCCI or EDCI, and in addition propanephosphonic anhydride (compare Angew. Chem. 92, 129 (1980)), diphenylphosphoryl azide or 2-ethoxy-N-ethoxycarbonyl-1,2-dihydroquinoline, in an inert solvent, e.g. a halogenated hydrocarbon such as dichloromethane, an ether such as tetrahydrofuran or dioxane, an amide such as DMF or dimethylacetamide, a nitrile such as acetonitrile, or in mixtures of these solvents, at temperatures between about -10 and 40, preferably between 0 and 30°.

Instead of II, suitable reactive derivatives of these substances can also be employed in the reaction, e.g. those in which reactive groups are intermediately blocked by protecting groups. The amino acid derivatives II can be used e.g. in the form of their activated esters which are expediently formed in situ, e.g. by addition of HOBt or N-hydroxysuccinimide.

The starting materials of the formula II are, as a rule, novel. They can be prepared by known methods, e.g. the abovementioned methods of peptide synthesis and of removal of protective groups.

As a rule, protected peptide esters of the formula R'-M'-OR'' are initially synthesized, where M' corresponds to the radical M reduced at the N-terminal end by an H atom, e.g. BOC-M'-OMe or Fmoc-M'-OMe. These are hydrolysed to acids of the formula R'-M'-OH, e.g. BOC-M'-OH or Fmoc-M'-OH and then condensed with a compound of the formula III, which is optionally likewise provided with corresponding protective groups at positions which should not be accessible to the reaction.

In the case of compounds of the formula III, peptide esters of the formula R'-Q-Z'-R'', such as e.g. BOC-Q-Z'-OMe or Fmoc-Q-Z'-OMe, are likewise synthesized, where Z' is -NH- or -O- and then, before the condensation for the preparation of compounds of the formula I is carried out, the protective group R' is cleaved in a known manner, e.g. Fmoc by treatment with a piperidine/DMF solution.

Particularly advantageously, the more recent

methods of peptide synthesis according to modified Merrifield techniques and using peptide synthesis apparatus as is described e.g. in Peptides, Proc. 8th Am. Pept. Symp., Eds. V. Hruby and D.H. Rich, Pierce Comp. III, pp. 73-77 (1983) by A. Jonczyk and J. Meienhofer (Fmoc strategy) or the techniques presented in Angew. Chem. 104, 375-391 (1992) can be used. Methods of this type are known per se and their description at this point is therefore unnecessary.

A base of the formula I can be converted into the appropriate acid addition salt using an acid. Suitable acids for this reaction are in particular those which yield physiologically acceptable salts. Inorganic acids can thus be used, e.g. sulfuric acid, nitric acid, hydrohalic acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such as orthophosphoric acid and sulfamic acid, and in addition organic acids, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic mono- or polybasic carboxylic, sulfonic or sulfuric acids, e.g. formic acid, acetic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, benzoic acid, salicylic acid, 2- or 3-phenylpropionic acid, citric acid, gluconic acid, ascorbic acid, nicotinic acid, isonicotinic acid, methane- or ethanesulfonic acid, ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalene-mono- and -disulfonic acids, and laurylsulfuric acid. Salts with physiologically unacceptable acids, e.g. picrates, can be used for the isolation and/or purification of the compounds of the formula I.

On the other hand, an acid of the formula I can be converted into one of its physiologically acceptable metal or ammonium salts by reaction with a base. Suitable salts here are in particular the sodium, potassium, magnesium, calcium and ammonium salts, and also substituted ammonium salts, e.g. the dimethyl-, diethyl- or diisopropylammonium salts, monoethanol-, diethanol- or

triethanolammonium salts, cyclohexyl- or dicyclohexylammonium salts, dibenzylethylenediammonium salts, and furthermore e.g. salts with N-methyl-D-glucamine or with arginine or lysine.

In addition, the novel compounds of the formula I can be used as integrin ligands for the preparation of columns for affinity chromatography for the preparation of integrins in pure form.

The ligand, i.e. a peptide derivative of the formula I, is in this case covalently coupled to a polymeric support via anchor functions.

Suitable polymeric support materials are the polymeric solid phases known per se in peptide chemistry, having preferably hydrophilic properties, for example crosslinked polysugars, such as cellulose, Sepharose or Sephadex®, acrylamides, polymers based on polyethylene glycol or Tentakel polymers®.

Suitable anchor functions which are linked to the polymeric supports are preferably linear alkylene chains having 2-12 C atoms, which are bonded directly to the polymer at one end and have a functional group, such as e.g. hydroxyl, amino, mercapto, maleimido or -COOH at the other end and are suitable to be linked to the C- or N-terminal section of the respective peptide.

It is possible in this case that the peptide be bonded directly or likewise via a second anchor function to the anchor of the polymer. It is also possible that peptides which contain amino acid radicals with functionalized side chains are bonded to the anchor function of the polymer via these.

Moreover, certain amino acid radicals which are a constituent of the peptides of the formula I can be modified in their side chains in such a way that they are available for anchorage via e.g. SH, OH, NH_2 or COOH groups with the anchor of the polymer.

In this connection, unusual amino acids are possible, such as e.g. phenylalanine derivatives which carry a mercapto, hydroxyl, amino or carboxyalkyl chain in position 4 of the phenyl ring, the functional group

being located at the end of the chain.

Examples of amino acid radicals whose side chain can be used directly as an anchor function are e.g. Lys, Orn, Arg, Dab, Asp, Asn, Glu, Gln, Ser, Thr, Cys, Cit or Tyr.

Examples of N-terminal anchors are radicals, such as e.g. $-CO-C_nH_{2n}-NH_2$, $-CO-C_nH_{2n}-OH$, $-CO-C_nH_{2n}-SH$ or $-CO-C_nH_{2n}-COOH$, where n = 2-12, the length of the alkylene chain not being critical and it optionally also being possible to replace this partially or completely e.g. by appropriate aryl or alkylaryl radicals.

C-terminal anchors can be, for example, $-O-C_nH_{2n}-SH$, $-O-C_nH_{2n}-OH$, $-O-C_nH_{2n}-NH_2$, $-O-C_nH_{2n}-COOH$, $-NH-C_nH_{2n}-SH$, $-NH-C_nH_{2n}-OH$, $-NH-C_nH_{2n}-NH_2$ or $-NH-C_nH_{2n}-COOH$, what has already been said in the preceding section applying to n and also to the alkylene chain.

The N- and C-terminal anchors can also be used as anchor components for an already functionalized side chain of an amino acid radical. Suitable amino acid radicals here, for example, are those such as Lys(CO-$C_5H_{10}-NH_2$), Asp(NH-C_3H_6-COOH) or Cys($C_3H_6-NH_2$), the anchor always being bonded to the functional group of the side chain.

The preparation of the materials for affinity chromatography is carried out under conditions such as are customary for the condensation of amino acids and are known per se and have already been outlined in the section for the preparation of the compounds of the formula I, or are described in Pierce, Immunotechnology Catalog & Handbook (1990)).

The novel compounds of the formula I and their physiologically acceptable salts can be used for the production of pharmaceutical preparations by bringing them into a suitable dosage form together with at least one excipient or auxiliary and, if desired, together with one or more other active compound(s). The preparations thus obtained can be employed as medicaments in human or veterinary medicine. Suitable excipient substances are organic or inorganic substances which are suitable for

enteral (e.g. oral or rectal), parenteral (e.g. intravenous injection) or local (e.g. topical, dermal, ophthalmic or nasal) administration or for administration in the form of an inhalant spray and which do not react with the novel compounds, for example water or aqueous isotonic saline solution, lower alcohols, vegetable oils, benzyl alcohols, polyethylene glycols, glycerol triacetate and other fatty acid glycerides, gelatin, soya lecithin, carbohydrates such as lactose or starch, magnesium stearate, talc, cellulose and petroleum jelly. Tablets, coated tablets, capsules, syrups, juices or drops, in particular, are used for oral administration; film tablets and capsules having enteric coatings or capsule shells are especially of interest. Suppositories are used for rectal administration, and solutions, preferably oily or aqueous solutions, and in addition suspensions, emulsions or implants, are used for parenteral administration. Solutions, e.g., which can be used in the form of eye drops, and in addition, e.g. suspensions, emulsions, creams, ointments or compresses are suitable for topical application. Sprays can be used which contain the active compound either dissolved or suspended in a propellant gas or propellant gas mixture (e.g. CO_2 or chlorofluorohydrocarbons) for administration as inhalant sprays. The active compound here is expediently used in micronized form, it being possible for one or more additional physiologically tolerable solvents to be present, e.g. ethanol. Inhalant solutions can be administered with the aid of customary inhalers. The novel compounds can also be lyophilized and the lyophilizates obtained used e.g. for the production of injection preparations. The injections can be administered as a bolus or as a continuous infusion (e.g. intravenous, intramuscular, subcutaneous or intrathecal). The preparations indicated can be sterilized and/or can contain auxiliaries such as preservatives, stabilizers and/or wetting agents, emulsifiers, salts for influencing osmotic pressure, buffer substances, colorants and/or flavourings. If desired, they can also contain one or

more other active compounds, e.g. one or more vitamins.

The substances according to the invention can as a rule be administered in analogy to other known commercially available peptides, but in particular in analogy to the compounds described in US-A-4,472,305, preferably in dosages between about 0.05 and 500 mg, in particular between 0.5 and 100 mg, per dosage unit. The daily dose is preferably between about 0.01 and 2 mg/kg of body weight. The specific dose for each intended patient depends, however, on many different factors, for example the activity of the specific compound employed, the age, body weight, general state of health, sex, the diet, the time and route of administration, and the rate of excretion, pharmaceutical combination and severity of the particular disorder to which the therapy applies. Parenteral administration is preferred.

All temperatures above and below are stated in °C. In the following examples, "customary working up" means: water is added, if necessary, the mixture is neutralized and extracted with ether or dichloromethane, the organic phase is separated off, dried over sodium sulfate, filtered and evaporated and the residue is purified by chromatography on silica gel and/or crystallization. RT = retention time (minutes) for HPLC on a Lichrosorb RP® select B (250-4.7 µm) column, eluent: 0.3 % TFA in water; isopropanol gradient from 0-80 % by vol. in 50 min at 1 ml/min. Flow and detection at 215 nm. M^+ = molecular peak in the mass spectrum, obtained by the fast atom bombardment method (FAB), as a rule is $M^+ + H$, i.e. the mass of the particular compound increased by 1 mass unit.

Example 1

2.2 g of BOC-Asp-Gly-OH are dissolved in a mixture of 150 ml of dichloromethane and 20 ml of DMF, cooled to 0° and then treated with 0.5 g of DCCI, 0.3 g of HOBt, 0.23 ml of N-methylmorpholine and 1 equivalent of H-Arg-His-Asp-Leu-OMe [both peptides are obtainable according to modified Merrifield technique methods]. The

reaction mixture is stirred for 20 hours at 0° and for 6 hours at room temperature. It is concentrated, treated with a mixed bed ion exchanger and added to an aqueous $NaHCO_3$ solution. The product which deposits is filtered off with suction and washed with water. Washing with ethyl acetate/petroleum ether gives BOC-Asp-Gly-Arg-His-Asp-Leu-OMe.

The following are obtained analogously by condensation of H-Arg-His-Asp-Leu-OMe

with BOC-Gly-OH:
BOC-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Asn-Gly-Asp-Gly-OH:
BOC-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Val-Asn-Gly-Asp-Gly-OH:
BOC-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Asp-Val-Asn-Gly-Asp-Gly-OH:
BOC-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Lys-Gly-Gly-Gly-Asp-Gly-OH:
BOC-Lys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Tyr-Gly-Gly-Gly-Asp-Gly-OH:
BOC-Tyr-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Ala-Asp-Gly-OH:
BOC-Ala-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-D-Ala-Asp-Gly-OH:
BOC-D-Ala-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Gly-Asp-Ala-OH:
BOC-Gly-Asp-Ala-Arg-His-Asp-Leu-OMe;

with BOC-Gly-D-Asp-Gly-OH:
BOC-Gly-D-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Gly-Asp-D-Ala-OH:
BOC-Gly-Asp-D-Ala-Arg-His-Asp-Leu-OMe;

with BOC-Gly-Asp-OH:
BOC-Gly-Asp-Arg-His-Asp-Leu-OMe;

with BOC-Cys(Trt)-Gly-Gly-Gly-Asp-OH:
BOC-Cys(Trt)-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OMe;

with BOC-Cys-Gly-Gly-Gly-Asp-OH:
BOC-Cys-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OMe;

with BOC-Cys(Trt)-Gly-Gly-Gly-Asp-Gly-OH:
BOC-Cys(Trt)-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Cys-Gly-Gly-Gly-Asp-Gly-OH:
BOC-Cys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Cys-Gly-Gly-Thr-Asp-Val-Asn-Gly-Asp-Gly-OH:
BOC-Cys-Gly-Gly-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe;

with BOC-Thr-Asp-Val-Asn-Gly-Asp-Gly-OH:
BOC-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe.

Example 2

The following are obtained analogously to

Example 1 by reaction of BOC-Gly-Asp-Gly-OH

with H-Arg-His-Asp-OMe:

BOC-Gly-Asp-Gly-Arg-His-Asp-OMe;

with H-Arg-His-OMe:

BOC-Gly-Asp-Gly-Arg-His-OMe;

with H-Arg-His-Asn-Leu-OMe:

BOC-Gly-Asp-Gly-Arg-His-Asn-Leu-OMe;

with H-Arg-Gly-Asp-Leu-OMe:

BOC-Gly-Asp-Gly-Arg-Gly-Asp-Leu-OMe;

with H-Arg-His-D-Asp-Leu-OMe:

BOC-Gly-Asp-Gly-Arg-His-D-Asp-Leu-OMe;

with H-Arg-D-His-Asp-Leu-OMe:

BOC-Gly-Asp-Gly-Arg-D-His-Asp-Leu-OMe;

with H-D-Arg-His-Asp-Leu-OMe:

BOC-Gly-Asp-Gly-D-Arg-His-Asp-Leu-OMe.

The following are obtained analogcusly to Example 1 by reaction of BOC-Gly-Asn-Gly-OH

with H-Arg-His-Asn-Leu-OMe:

BOC-Gly-Asn-Gly-Arg-His-Asn-Leu-OMe;

with H-Arg-His-Asp-Leu-OMe:

BOC-Gly-Asn-Gly-Arg-His-Asp-Leu-OMe.

Example 3

The following are obtained analogously to Example 1 by condensation of:

BOC-Leu-Asp-His-Arg-OH with H-Gly-Asp-Gly-OEt:

BOC-Leu-Asp-His-Arg-Gly-Asp-Gly-OEt;

BOC-Lys-Gly-Gly-Gly-Asp-Arg-Leu-OH with H-His-Asp-Gly-OEt:

BOC-Lys-Gly-Gly-Gly-Asp-Arg-Leu-His-Asp-Gly-OEt;

BOC-Lys-Pro-Ser-Asp-OH with H-Gly-Arg-Gly-OEt:

BOC-Lys-Pro-Ser-Asp-Gly-Arg-Gly-OEt;

BOC-Arg-His-OH with H-Asp-Leu-OMe:

BOC-Arg-His-Asp-Leu-OMe;

BOC-D-Lys-D-Pro-D-Ser-D-Asp-D-Gly-OH with H-D-Arg-D-Gly-OMe:

BOC-D-Lys-D-Pro-D-Ser-D-Asp-D-Gly-D-Arg-D-Gly-OMe;

BOC-Leu-Asp-His-OH with H-Arg-Gly-Asp-OMe:

BOC-Leu-Asp-His-Arg-Gly-Asp-OMe;

BOC-Leu-Asp-His-OH with H-Arg-Gly-OMe:

BOC-Leu-Asp-His-Arg-Gly-OMe;

BOC-Gly-Arg-His-OH with H-Asp-Leu-Leu-OMe:

BOC-Gly-Arg-His-Asp-Leu-Leu-OMe;

BOC-Arg-Gly-OH with H-Asp-Leu-OMe:

BOC-Arg-Gly-Asp-Leu-OMe;

BOC-Cys-Gly-Gly-Gly-Asp-Arg-OH with H-Leu-His-Gly-OMe:

BOC-Cys-Gly-Gly-Gly-Asp-Arg-Leu-His-Gly-OMe;

BOC-Cys-Gly-Gly-Gly-Asp-Gly-Arg-OH with H-His-Asp-Ile-OMe:

BOC-Cys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Ile-OMe.

Example 4

1.3 g of BOC-Asp-Gly-Arg-His-Asp-Leu-OMe are dissolved in 60 ml of methanol, treated with 1.5 ml of 2 N NaOH solution and stirred for 4 hours at 25°. After removal of the solvent, the residue is taken up in water, the pH is adjusted to 3 by the addition of dilute HCl and the mixture is extracted with ethyl acetate. The extract is dried over Na_2SO_4. Removal of the solvent gives BOC-Asp-Gly-Arg-His-Asp-Leu-OH, which is taken up in 20 ml of 2 N HCl in dioxane and stirred for 2 hours at room temperature. The reaction mixture is concentrated to dryness and the residue purified by HPLC. H-Asp-Gly-Arg-His-Asp-Leu-OH is obtained; RT = 9.5; M^+ 712.

The following are obtained analogously by removal of the protective groups, starting from the compounds from Example 1:

H-Gly-Arg-His-Asp-Leu-OH, RT = 9.2; M^+ 597;

H-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 11.3; M^+ 883;

H-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 12.9; M^+ 982;

H-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 13.4; M^+ 1097;

H-Lys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 11.5; M^+ 1011;

H-Tyr-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 13.8; M^+ 1046;

H-Ala-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 10.7; M^+ 783;

H-D-Ala-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 11.1; M^+ 783;

H-Gly-Asp-Ala-Arg-His-Asp-Leu-OH; RT = 11.0; M^+ 784;

H-Gly-D-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 10.5; M^+ 770;

H-Gly-Asp-D-Ala-Arg-His-Asp-Leu-OH; RT = 11.9; M^+ 784;

H-Gly-Asp-Arg-His-Asp-Leu-OH; RT = 8.1; M^+ 712;

H-Cys(Trt)-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH; RT = 27.7; M^+ 1171;

H-Cys-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH, RT = 11.7, M^+ 929,

H-Cys(Trt)-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH, RT = 27, M^+ 1228;

H-Cys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 11.7; M^+ 987;

H-Cys-Gly-Gly-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH, RT = 12 7; M^+ 1415;

H-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH.

Example 5

The following are obtained analogously to Example 4 by hydrolysis and removal of the BOC protective groups, starting from the compounds from Example 2:

H-Gly-Asp-Gly-Arg-His-Asp-OH; RT = 3.5, M^+ 656;

H-Gly-Asp-Gly-Arg-His-OH; RT = 3.5; M^+ 541;

H-Gly-Asp-Gly-Arg-His-Asn-Leu-OH; RT = 11.2; M^+ 769;

H-Gly-Asp-Gly-Arg-Gly-Asp-Leu-OH; RT = 11.4; M^+ 689;

H-Gly-Asp-Gly-Arg-His-D-Asp-Leu-OH; RT = 11.7; M^+ 769;

H-Gly-Asp-Gly-Arg-D-His-Asp-Leu-OH; RT = 11.3; M^+ 769;

H-Gly-Asp-Gly-D-Arg-His-Asp-Leu-OH; RT = 10.9; M^+ 769.

H-Gly-Asn-Gly-Arg-His-Asn-Leu-OH; RT = 10.8; M^+ 767;

H-Gly-Asn-Gly-Arg-His-Asp-Leu-OH, RT = 11 6, M^+ 768.

Example 6

The following are obtained analogously to Example 4 starting from the compounds from Example 3 by hydrolysis and removal of the BOC protective groups:

H-Leu-Asp-His-Arg-Gly-Asp-Gly-OH; RT = 11.1; M^+ 769;

H-Lys-Gly-Gly-Gly-Asp-Arg-Leu-His-Asp-Gly-OH; RT = 9 8; M^+ 1011;

H-Lys-Pro-Ser-Asp-Gly-Arg-Gly-OH, RT = 3.5; M^+ 716;

H-Arg-His-Asp-Leu-OH; RT = 8 0; M^+ 540;

H-D-Lys-D-Pro-D-Ser-D-Asp-D-Gly-D-Arg-D-Gly-OH, RT = 24.7; M^+ 938;

H-Leu-Asp-His-Arg-Gly-Asp-OH; RT = 11.5; M^+ 712;

H-Leu-Asp-His-Arg-Gly-OH; RT = 3.9; M^+ 597;

H-Gly-Arg-His-Asp-Leu-Leu-OH; RT = 15.3; M^+ 710;

H-Arg-Gly-Asp-Leu-OH; RT = 7.0; M^+ 460;

H-Cys-Gly-Gly-Gly-Asp-Arg-Leu-His-Gly-OH; RT = 7.2; M^+ 871;

H-Cys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Ile-OH; RT = 15.1;

M^+ 986.

Example 7

0.9 g of H-Arg-His-Asp-Leu-OH is dissolved in 200 ml of aqueous DMF and treated dropwise with stirring with 0.5 g of acetyl chloride, dissolved in 10 ml of dichloromethane. The reaction mixture is stirred for 15 minutes and strongly concentrated. The product which deposits is separated off. H_3C-CO-Arg-His-Asp-Leu-OH is obtained; RT = 12.4; M^+ 582.

The following are obtained by acetylation

of H-Gly-Asp-Gly-Arg-His-OH:

H_3C-CO-Gly-Asp-Gly-Arg-His-OH;

of H-Arg-Gly-Asp-Leu-OH:

H_3C-CO-Arg-Gly-Asp-Leu-OH; RT = 13.0; M^+ 502;

of H-Lys-Pro-Ser-Asp-Gly-Arg-Gly-OH:

H_3C-CO-Lys-Pro-Ser-Asp-Gly-Arg-Gly-OH

of H-Arg-His-Asp-Leu-OH:

H_3C-CO-Arg-His-Asp-Leu-OH;

of H-D-Lys-D-Pro-D-Ser-D-Asp-D-Gly-D-Arg-D-Gly-OH:

H_3C-CO-D-Lys-D-Pro-D-Ser-D-Asp-D-Gly-D-Arg-D-Gly-OH;

of H-Leu-Asp-His-Arg-Gly-Asp-OH:

H_3C-CO-Leu-Asp-His-Arg-Gly-Asp-OH;

of H-Leu-Asp-His-Arg-Gly-OH:

H_3C-CO-Leu-Asp-His-Arg-Gly-OH.

Example 8

Analogously to Example 1, condensation of H_3C-CO-Gly-Asp-Gly-Arg-His-OH with H-Asp-Leu-OMe and subsequent hydrolysis gives H_3C-CO-Gly-Asp-Gly-Arg-His-Asp-Leu-OH; RT = 14.1; M$^+$ 811.

Example 9

2.0 g of BOC-Gly-Arg-His-Asp-Leu-OMe are stirred for two hours in 25 ml of 4 N hydrochloric acid in dioxane. The reaction mixture is then concentrated, the residue is dissolved in 100 ml of DMF and the solution is cooled to 0°. 1 equivalent of Fmoc-Gly-Gly-Gly-Asp-OH, 1.3 g of TBTU and 1.0 ml of triethylamine are then added successively. The solution is stirred for 2 hours at 0° and for 12 hours at room temperature. After fresh concentration, the concentrate is poured into an $NaHCO_3$ solution. The product which deposits during the course of this is filtered off and dissolved in 50 ml of methanol, and the solution is treated with 1.5 ml of 2 N NaOH solution, stirred for 4 hours at 25° and worked up in the customary manner. Fmoc-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH is obtained;
RT = 28.0; M$^+$ 1105.

The following are obtained analogously by condensation of H-Arg-His-Asp-Leu-OMe

with Fmoc-Gly-OH:

Fmoc-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Asn-Gly-Asp-Gly-OH:
Fmoc-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Val-Asn-Gly-Asp-Gly-OH:
Fmoc-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Asp-Val-Asn-Gly-Asp-Gly-OH:
Fmoc-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Lys-Gly-Gly-Gly-Asp-Gly-OH:
Fmoc-Lys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Tyr-Gly-Gly-Gly-Asp-Gly-OH:
Fmoc-Tyr-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Ala-Asp-Gly-OH:
Fmoc-Ala-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-D-Ala-Asp-Gly-OH:
Fmoc-D-Ala-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Gly-Asp-Ala-OH:
Fmoc-Gly-Asp-Ala-Arg-His-Asp-Leu-OH;

with Fmoc-Gly-D-Asp-Gly-OH:
Fmoc-Gly-D-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Gly-Asp-D-Ala-OH:
Fmoc-Gly-Asp-D-Ala-Arg-His-Asp-Leu-OH;

with Fmoc-Gly-Asp-OH:
Fmoc-Gly-Asp-Arg-His-Asp-Leu-OH;

with Fmoc-Cys(Trt)-Gly-Gly-Gly-Asp-OH:
Fmoc-Cys(Trt)-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH;

with Fmoc-Cys-Gly-Gly-Gly-Asp-OH:
Fmoc-Cys-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH;

with Fmoc-Cys(Trt)-Gly-Gly-Gly-Asp-Gly-OH:
Fmoc-Cys(Trt)-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Cys-Gly-Gly-Gly-Asp-Gly-OH:
Fmoc-Cys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Cys-Gly-Gly-Thr-Asp-Val-Asn-Gly-Asp-Gly-OH:
Fmoc-Cys-Gly-Gly-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

with Fmoc-Thr-Asp-Val-Asn-Gly-Asp-Gly-OH:
Fmoc-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH.

Example 10

0.7 g of BOC-Gly-Asp-Gly-Arg(BOC)-His-Asp-Leu-OH is dissolved in 100 ml of dichloromethane, treated with 1.4 equivalents of MBHA resin and stirred for 24 hours at room temperature. Removal of the solvent gives BOC-Gly-Asp-Gly-Arg(BOC)-His-Asp-Leu-MBHA resin, which is taken up in 20 ml of 2 N HCl in dioxane and stirred for 2 hours at room temperature. Subsequent treatment with TFA yields H-Gly-Asp-Gly-Arg-His-Asp-Leu-NH_2; RT = 8.8; M^+ 768.

The following peptide amides are obtained analogously by reaction with MBHA resin:

from BOC-Arg(BOC)-His-Asp-Leu-OH:
H-Arg-His-Asp-Leu-NH_2; RT = 5.4; M^+ 539;

from BOC-Arg(BOC)-Gly-Asp-Leu-OH:

H-Arg-Gly-Asp-Leu-NH_2; RT = 4.7; M^+ 459;

from BOC-Gly-Asp-Gly-Arg(BOC)-Gly-Asp-Leu-OH:

H-Gly-Asp-Gly-Arg-Gly-Asp-Leu-NH_2.

Example 11

Analogously to Example 7, starting from H-Gly-Asp-Gly-Arg-His-Asp-Leu-NH_2, H_3C-CO-Gly-Asp-Gly-Arg-His-Asp-Leu-NH_2 is obtained by acetylation of the peptide; RT = 12.4; M^+ 810.

The following are obtained analogously:

from H-Arg-His-Asp-Leu-NH_2:

H_3C-CO-Arg-His-Asp-Leu-NH_2, RT = 11.1; M^+ 581;

from H-Arg-Gly-Asp-Leu-NH_2:

H_3C-CO-Arg-Gly-Asp-Leu-NH_2; RT = 11.3; M^+ 501.

Example 12

80 mg of H-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH are dissolved three to four times in 0.01 m HCl and freeze-dried after each dissolving operation. Subsequent purification by HPLC gives H-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH × HCl.

The following is obtained analogously

from H-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH by treatment with TFA:

H-Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu-OH x TFA;
RT = 11.8; M+ 1196.

Example 13

To prepare affinity phases, 0.9 g of N-maleimido-C_5H_{10}-CO-NH-C_3H_6-polymer [obtainable by condensation of N-maleimido-C_5H_{10}-COOH with H_2N-C_3H_6-polymer] is suspended in 10 ml of 0.1 M sodium phosphate buffer at pH 7 and 1 equivalent of H-Cys-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH is added at 4°. The mixture is stirred for 4 hours with simultaneous warming of the reaction mixture to room temperature, and the solid residue is filtered off and washed twice with 10 ml each of buffer solution (pH 7) and then three times with 10 ml each of water. H-Cys[3-(N-maleimido-C_5H_{10}-CO-NH-C_3H_6-polymer)]-Gly-Gly-Gly-Asp-Arg-His-Asp-Leu-OH is obtained.

Example 14

Analogously to Example 1, the following polymeric phase is obtained by condensation of polymer-O-C_3H_6-NH_2 [commercially available] and HOOC-C_4H_8-CO-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe [obtainable by condensation of adipic acid with H-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe under the conditions mentioned]:
Polymer-O-C_3H_6-NH-CO-C_4H_8-CO-Gly-Asp-Gly-Arg-His-Asp-Leu-OMe. From this, hydrolysis in methanol using 2 N NaOH solution according to Ex.4 gives polymer-O-C_3H_6-NH-CO-C_4H_8-CO-Gly-Ap-Gly-Arg-His-Asp-Leu-OH.

The examples below relate to pharmaceutical preparations.

Example A: Injection vials

A solution of 100 g of an active compound of the formula I and 5 g of disodium hydrogenphosphate in 3 l of doubly distilled water is adjusted to pH 6.5 with 2 N hydrochloric acid, sterile filtered, filled into injection vials and lyophilized under sterile conditions, and the vials are sealed in a sterile manner. Each

injection vial contains 5 mg of active compound.

Example B: Suppositories

A mixture of 20 g of an active compound of the formula I is fused with 100 g of soya lecithin and 1400 g of cocoa butter, and the mixture is poured into moulds and allowed to cool. Each suppository contains 20 mg of active compound.

Example C: Solution

A solution of 1 g of an active compound of the formula I, 9.38 g of $NaH_2PO_4 \cdot 2H_2O$, 28.48 g of $Na_2HPO_4 \cdot 12H_2O$ and 0.1 g of benzalkonium chloride is prepared in 940 ml of doubly distilled water. The solution is adjusted to pH 6.8, made up to 1 l and sterilized by irradiation. This solution can be used in the form of eye drops.

Example D: Ointment

500 mg of an active compound of the formula I are mixed with 99.5 g of petroleum jelly under aseptic conditions.

Example E: Tablets

A mixture of 1 kg of active compound of the formula I, 4 kg of lactose, 1.2 kg of potato starch, 0.2 kg of talc and 0.1 kg of magnesium stearate is pressed to give ta lets in a customary manner, such that each tablet contains 10 mg of active compound.

Example F: Coated tablets

Tablets are pressed analogously to Example E and then coated in a customary manner with a coating of sucrose, potato starch, talc, tragacanth and colorant.

Example G: Capsules

Hard gelatin capsules are filled with 2 kg of active compound of the formula I in the customary manner, such that each capsule contains 20 mg of active compound.

Example H: Ampoules

A solution of 1 kg of active compound of the formula I in 60 l of doubly distilled water is sterile filtered, filled into ampoules, lyophilized under sterile conditions and the ampoules are sealed in a sterile manner. Each ampoule contains 10 mg of active compound.

The Claims defining the invention are as follows:

1. Linear peptides of the formula I

X-A-B-C-Arg-E-G-L-Z I,

in which

X is H, acyl having 1-10 C atoms, H-Asn, H-Val-Asn, H-Asp-Val-Asn, Fmoc-Gly-Gly, H-Lys-Gly-Gly, H-Lys-Pro, H-Tyr-Gly-Gly, H-Cys-Gly-Gly, H-Cys(Trt)-Gly-Gly, H-Cys-Gly-Gly-Thr-Asp-Val-Asn or H-Thr-Asp-Val-Asn,

A, B and C in each case independently of one another are absent or are in each case an amino acid radical, selected from a group consisting of Ala, Arg, Asn, Asp, Asp(OR), Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Lys(Ac), Lys($AcNH_2$), Lys(AcSH), Met, Orn, Phe, 4-Hal-Phe, Pro, Ser, Thr, Trp, Tyr or Val, where the amino acid radicals mentioned can also be derivatized,

E is Gly, His or Leu-His,

G is absent or is Asp or Asn,

L is absent or is Gly, Ile, Leu or Leu-Leu,

Z is NH_2 or OH,

Hal is F, Cl, Br or I and

Ac is alkanoyl having 1-10 C atoms,

and their physiologically acceptable salts.

2. An enantiomer or a diasteromer of a compound of the formula I according to Claim 1.

3. (a) H-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

(b) H-Thr-Asp-Val-Asp-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

(c) H-Lys-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

(d) Fmoc-Gly-Gly-Gly-Asp-Gly-Arg-His-Asp-Leu-OH;

(e) CH3-CO-Gly-Asp-Gly-Arg-His-Asp-Leu-OH.

(f) H-Cys-Gly-Gly-Asp-Arg-His-Asp-Leu-OH;

(g) H-Gly-Arg-His-Asp-Leu-OH.

4. Process for the preparation of a compound of the formula I according to Claim 1 or of one of its salts, characterized in that it is liberated from one of its functional derivatives by treating with a solvolysing or hydrogenolysing agent

or in that a compound of the formula II

M-Z II,

in which

Z has the meaning indicated and

M is (a) X, but not hydrogen, Fmoc-Gly, H-Lys-Gly, H-Lys, H-Tyr-Gly, H-Asp-Val, H-Tyr, H-Val, H-Asp, H-Cys-Gly-Gly-Thr-Asp-Val, H-Cys-Gly-Gly-Thr-Asp, H-Cys-Gly-Gly-Thr, H-Cys-Gly-Gly, H-Cys-Gly, H-Cys, H-Cys(Trt)-Gly, H-Cys(Trt), H-Thr-Asp-Val, H-Thr-Asp or H-Thr,

(b) X-A,

(c) X-A-B,

(d) X-A-B-C,

(e) X-A-B-C-Arg,

(f) X-A-B-C-Arg-E or

(g) X-A-B-C-Arg-E-G

is reacted with an amino compound of the formula III

H-Q-Z III

in which

Z has the meaning indicated and

Q is (a) H-A-B-C-Arg-E-G-L, H-Asn-A-B-C-Arg-E-G-L, H-Val-Asn-A-B-C-Arg-E-G-L, H-Pro-A-B-C-Arg-E-G-L, H-Gly-A-B-C-Arg-E-G-L, H-Gly-Gly-A-B-C-Arg-E-G-L, H-Asn-A-B-C-Arg-E-G-L, H-Val-Asn-A-B-C-Arg-E-G-L, H-Asp-Val-Asn-A-B-C-Arg-E-G-L, H-Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L, H-Gly-Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L or H-Gly-Gly-Thr-Asp-Val-Asn-A-B-C-Arg-E-G-L,

(b) H-B-C-Arg-E-G-L,

(c) H-C-Arg-E-G-L,

(d) H-Arg-E-G-L,

(e) H-E-G-L,

(f) H-G-L or

(g) H-L

and/or in that a free amino group is optionally acylated and/or a compound of the formula I is converted into one of its salts by treating with an acid or a base.

5. Process for the production of pharmaceutical preparations, characterized in that a compound of the formula I according to Claim 1 and/or of one of its physiologically acceptable salts is brought into a suitable dosage form together with at least one solid, liquid or semi-liquid excipient or auxiliary.

6. Pharmaceutical preparation, characterized in that it contains at least one compound of the general formula I according to Claim 1 and/or one of its physiologically acceptable salts.

7. Use of compounds of the formula I according to Claim 1 or of their physiologically acceptable salts for the production of a medicament for the control of diseases.

8. Use of compounds of the formula I according to Claim 1 or of their physiologically acceptable salts in the control of diseases.

9. Use of compounds of the formula I according to Claim 1 for the preparation of immobilized ligands for affinity column chromatography.

10. Use of compounds of the formula I according to Claim 1 for the purification of integrins by affinity chromatography.

11. A Compound as claimed in any one of Claims 1 to 8, methods for their manufacture or pharmaceutical compositions or methods of treatment involving/ containing them, substantially as hereinbefore described with reference to the Examples.

12. A method for the treatment of disease which comprises administering to a subject in need of such treatment a therapeutically effective amount of a compound of the formula I optionally in association with a pharmaceutically acceptable carrier.

DATED this 22nd day of March 1994,

MERCK PATENT GMBH
By Its Patent Attorney
DAVIES COLLISON CAVE

Abstract

The invention relates to novel linear peptides of the formula I

X-A-B-C-Arg-E-G-L-Z I,

in which A, B, C, E, G, L, X and Z have the meaning indicated in Claim 1, and their salts.

These compounds act as integrin inhibitors and can be used in particular for the prophylaxis and treatment of disorders of the circulation and for tumour therapy.